

Capillarity-like growth of protein folding nuclei

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Abstract

A full structural description of transition state ensembles in protein folding includes the specificity of the ordered residues composing the folding nucleus as well as spatial density. To our knowledge, the spatial properties of the folding nucleus and interface of specific nuclei has yet to receive significant attention. We analyzed folding routes predicted by a variational model in terms of a generalized formalism of the capillarity scaling theory that assumes the volume of the folded core of the nucleus grows with chainlength as $V_f \sim N^{3\nu}$. For 28 two-state proteins studied, the scaling exponent ν ranges from 0.2 to 0.45 with an average of 0.33. This average value corresponds to packing of rigid objects, though generally the effective monomer size in the folded core is larger than the corresponding volume per particle in the native state ensemble. That is, on average the folded core of the nucleus is found to be relatively diffuse. We also studied the growth of the folding nucleus and interface along the folding route in terms of the density or packing fraction. The evolution of the folded core and interface regions can be classified into three patterns of growth depending on how the growth of the folded core is balanced by changes in density of the interface. Finally, we quantify the diffuse versus polarized structure of the critical nucleus through direct calculation of the packing fraction of the folded core and interface regions. Our results support the general picture of describing protein folding as the capillarity-like growth of folding nuclei.

The modern theory of protein folding describes the mechanism for folding as an entropic bottleneck arising from the decreasing number of accessible pathways available to a protein as it becomes ordered.^{1,2} The collection of partially ordered conformations corresponding to this bottleneck region is known as the transition state ensemble or critical folding nucleus.³ Although it is common to focus on the degree of native-like order of specific residues, a complete description of the protein folding mechanism also includes the spatial properties such as size or density of the transition state ensemble. Indeed, shortly after characterizing the transition state ensemble of CI2, Fersht summarized the structure of the critical nucleus by a spatial description through the proposal of the *nucleation-condensation* mechanism.⁴ This critical nucleus can be thought of as an expanded, partially ordered version of the native state ensemble with concomitant longranged tertiary and local secondary structure. It is now clear that while diffuse nuclei appear to be the general rule, some nuclei are less diffuse than others.⁵ Polarized nuclei have highly structured residues which are spatially clustered in the native structure, while the rest of the residues show little definite order.^{6,7,8,9} Such nuclei are similar to the capillarity approximation in homogeneous nucleation in which the free energy of a stable phase droplet is separated from the metastable phase by a very sharp interface.^{10,11} Exploiting this analogy, Wolynes describes a nucleus with capillarity-like order in which the interface surrounding a relatively folded core is broadened by wetting of partially ordered residues.¹¹ In this picture, folding can be described as the growth of the folding nucleus: a wave of order moving across the protein as the edge of the nucleus expands to ultimately consume the entire molecule.^{11,12}

The extended partially ordered interface of a capillarity-like ordered nucleus separates space into three regions: a folded core, a partially ordered interface region, and unfolded halo (see Fig. 1). In this paper, we monitor the structural development of the nucleus along the folding route through the evolution of the packing fraction of the folded core and the interface. As shown in Fig. 1, growth of the nucleus can be described by fluxes of residues passing through two moving surfaces: one surface separates the folded core and interface, and the other surface separates the interface region and the unfolded halo. As the protein folds, the evolution of the interface is determined by the interfacial volume and the net flux of residues entering the interface.

Our analysis is based on folding routes calculated for 28 two-state proteins from a cooperative variational model described in¹³. We note this model includes neutral cooperativity

due to repulsive excluded volume interactions. This form of cooperativity has been shown to broaden the range of barrier heights allowing direct comparison between calculated and measured folding rates.¹³ Not surprisingly, cooperativity tends to sharpen the interface between folded and unfolded regions. Nevertheless, the interface from this model is generally not nearly as sharp as a strict capillarity description in which a residue can be clearly identified as being either completely folded or completely unfolded as some other analytic models assume.^{14,15,16} In fact, an unbiased analysis of the spatial properties of the folding nucleus fundamentally depends on the model's ability to describe partial order.

Capillarity-like growth of folding nucleus

Capillarity picture of folding nuclei. The capillarity approximation of folding nuclei is based on classical nucleation theory of first order phase transition kinetics.^{11,17} Within the capillarity approximation, the free energy of a nucleus with volume V_f and surface area A_f can be written as a sum of two terms

$$F = -\Delta f V_f + \gamma A_f, \quad (1)$$

where Δf denotes the bulk free energy difference per unit volume between the unfolded and folded ensembles, and γ is the surface tension between the folded and unfolded regions.

A folded core with native-like density has a volume per monomer independent of its size. Relaxing this assumption, we assume that the number of residues in the folded core, N_f , scales with its volume, V_f , according to

$$V_f = b^3 N_f^{3\nu}. \quad (2)$$

Here, ν is the scaling exponent associated with the lengthscale of the folded core $R \sim b_0 N_f^\nu$, and b^3 is a geometry dependent elementary volume proportional to the monomer volume, b_0^3 . The free energy of a folded nucleus with N_f residues then has the form:¹¹

$$F(N_f) = -\Delta f b^3 N_f^{3\nu} + \gamma b^2 N_f^{2\nu}, \quad (3)$$

At the folding transition temperature, T_f , finite size depression of the surface energy suggests that $\gamma \sim \Delta f b N^\nu$ where N is the number of monomers in the protein. The maximum of the free energy occurs at $N_f^\dagger = (2/3)^{1/\nu} N$, giving the size of the critical nucleus, and the

associated free energy barrier scales as $\Delta F^\dagger \sim N^{2\nu}$. If we assume that the folded core has native like packing, $\nu = 1/3$ and b^3 is the native-like volume per monomer, so that $N_f^\dagger = (2/3)^3 N$ and $\Delta F^\dagger \sim N^{2/3}$.^{10,11}

Simulations and alternative theoretical considerations also suggest that barrier height (logarithm of the folding time) scales sublinearly on chainlength, $\Delta F^\dagger \sim N^p$ with $0 \leq p \leq 1$.^{10,11,18,19} Direct analysis of folding rate data to determine the scaling exponent p encounters the difficulty that the range of N is too small to distinguish between different values of p .^{20,21,22,23,24} So while it may be reasonable to expect that the scaling of the barrier height with chainlength is universal for sufficiently large proteins, the size of a typical two state proteins (~ 100 amino acids) may well be too small to be governed by this generic behavior. In this case, both specificity and size of these smaller proteins should generally determine the properties of the critical nuclei. In this paper, we assume that Eq. 2 is valid to describe the growth of the nucleus in all the two state proteins, but the exponent ν and volume b^3 are allowed to be protein specific.

Characterizing the folded core and the interface. In the variational model considered in this paper, partially ordered configurations are described by a variational Hamiltonian, \mathcal{H}_0 , corresponding to a stiff polymer chain inhomogeneously constrained to the native structure. Since this model is described in detail in Ref.¹³, we focus here on how to define folded core, interface, and unfolded regions along the calculated folding route. This is not as straight-forward as one might expect because the concept directly couples specificity of the nucleus with the spatial density.

We characterize the degree of structure of each residue by the extent of localization about the native structure $\{\mathbf{r}^N\}$, $\rho_i = \langle \exp(-\alpha^N(\mathbf{r}_i - \mathbf{r}_i^N)^2) \rangle_0$, with $\alpha^N = 0.1$. Here, the subscript denotes the average with respect to the Boltzmann weight with \mathcal{H}_0 . Denoting the native density at the globule and native state by $\rho_i(\text{G})$ and $\rho_i(\text{N})$, respectively, we consider the normalized density

$$\tilde{\rho}_i = \frac{\rho_i - \rho_i(\text{G})}{\rho_i(\text{N}) - \rho_i(\text{G})} \quad (4)$$

as a set of order parameters characterizing the folding of each residue. Progress along the folding route can be monitored by the global structural parameter $Q = 1/N \sum \tilde{\rho}_i$.

The normalized native densities are used to define a fiducial set of folded residues, $\{F\}$, with $\tilde{\rho}_i > 0.6$, as shown in Fig. 2a. Next, we define the spatial region of the folded core

through the relative contribution of the density of the folded residues in $\{F\}$, $n_f(\mathbf{r}) = \sum_{\{F\}} \langle \delta(\mathbf{r} - \mathbf{r}_i) \rangle_0$, to the total density, $n(\mathbf{r}) = \sum_{i=1}^N \langle \delta(\mathbf{r} - \mathbf{r}_i) \rangle_0$. The spatial extent of the folded core and interfacial regions in this analysis is determined by an indicator function

$$\tilde{n}(\mathbf{r}) = \frac{n_f(\mathbf{r})}{n(\mathbf{r})}. \quad (5)$$

which ranges from $0 \leq \tilde{n}(\mathbf{r}) \leq 1$. We define the folded core region, \mathcal{V}_f , as the points $\{\mathbf{r}_f\}$ for which the density of the fiducial folded residues contributes at least 50% to the total density ($\tilde{n}(\mathbf{r}) \geq 0.5$). The number of residues in this folded core region can be found by numerically integrating the density over the core region, $N_f = \int_{\mathcal{V}_f} n(\mathbf{r}) d\mathbf{r}$. The volume of the core region is given by $V_f = \int_{\mathcal{V}_f} d\mathbf{r}$.

Similarly, the interfacial region, \mathcal{V}_{int} , is defined as the points $\{\mathbf{r}_{\text{int}}\}$ for which $0.1 \leq \tilde{n}(\mathbf{r}_{\text{int}}) < 0.5$. The number of interfacial residues and volume of the interface is given by $N_{\text{int}} = \int_{\mathcal{V}_{\text{int}}} n(\mathbf{r}) d\mathbf{r}$, and $V_{\text{int}} = \int_{\mathcal{V}_{\text{int}}} d\mathbf{r}$, respectively.

The number of residues and the volume can be used to define a mean packing fraction of the folded core and partially ordered interface by

$$\mu_f = \frac{N_f}{V_f} v_0 \quad \text{and} \quad \mu_{\text{int}} = \frac{N_{\text{int}}}{V_{\text{int}}} v_0, \quad (6)$$

respectively. Here, v_0 is the calculated volume of per particle of the native structure at the folding transition temperature, T_f . The growth of the nucleus can be characterized by the way the packing fractions μ_f and μ_i change along the folding route.

Growth of folding nucleus along the folding route. As illustrated in Fig.2(a-b), the changes in N_f and V_f along a folding route can be fit to Eq. 2 to give an estimate of the scaling exponent ν for each protein. Fig. 2(c) shows the distribution of predicted ν from the folding routes obtained from the variational model for 28 two-state proteins discussed in ¹³. The predicted scaling exponent ν ranges between $0.2 \sim 0.42$ with an average of $\nu = 0.33$. The mean exponent is very close to the the scaling associated with close-packed rigid objects, $\nu = 1/3$. For comparison, recent detailed statistical models indicate that the scaling exponent for the unfolded state of a protein is about ²⁵ $\nu = 0.59$, while the folded state of a wide variety of proteins suggest that proteins with less than 300 amino acids have compact folded structures ($\nu = 0.3$), while larger proteins are less dense ($\nu = 0.4$).²⁶

The mean packing fraction of the core scales with the number of monomers as:

$$\mu_f = \frac{v_0}{b^3} N_f^{1-3\nu}. \quad (7)$$

For the close packing value $\nu = 1/3$, μ_f is independent of the number of monomers and the core retains native like density as it grows. When $\nu > 1/3$, the core becomes less compact as monomers are added to the core. This is the familiar scaling from loosely packed or fractal objects. When $\nu < 1/3$, the core density increases as more monomers are incorporated into the core. This can be understood as the consolidation of structure in the folded core as folding progresses.

Although the spatial structure of the critical folding nucleus (transition state ensemble) is discussed in more detail later, it is instructive to consider the value of the mean packing fraction of the core here. Fig. 2d shows the distribution of packing fractions of the folded core evaluated at the maximum free energy barrier between folded and unfolded states at T_f . The packing fraction has a wide range from 0.2 to 1.0. While some of the transition state nuclei have compact cores, the average packing fraction is only 0.59. This means that although the growth of a typical folded core corresponds to rigidly packed objects, a typical transition state ensemble has a folded core with twice the volume as the volume of same number of monomers in the native state conformation ($b^3 \approx 2v_0$). That is, the monomers composing the nucleus are typically much less localized than in the native state.

Fig. 3 illustrates a typical example of the growth of the folded core and the interface region. Early in the folding, we see a small compact nucleus surrounded by a partially folded interface. This small nucleus is partially ordered, occupying about twice the volume as the corresponding residues in the native state. Structural fluctuations giving nuclei corresponding to $Q < Q^\dagger$ are unstable with respect to the unfolded state due to relatively large surface free energy cost associated with small nuclei, whereas structural fluctuations with $Q > Q^\dagger$ will tend to evolve to the folded state. As the nucleus grows, the volume of the nucleus evolves as interfacial regions are incorporated into the core, while unfolded regions become part of the partially ordered interface.

Growth patterns of the nucleus: The structural growth of the folding nucleus can be understood as the competition between growth of the folded core and the evolution of the interface. The flux of residues entering core through the interface region controls the growth

of the core, while the net flux of residues entering interface region from the unfolded halo controls the growth of the interface (see Fig. 1). We characterize the evolution of the structure of the folding by the changes in the packing fraction of the core and interface regions as a function of Q . That is, we consider the signs of

$$\dot{\mu}_f(Q) = \frac{d\mu_f}{dQ} \quad \text{and} \quad \dot{\mu}_{\text{int}}(Q) = \frac{d\mu_{\text{int}}}{dQ} \quad (8)$$

to identify different modes of growth. From the two state proteins used in this study, we can identify three distinct scenarios as illustrated in Fig. 4.

- Pattern A (*consolidation of core and interface*). As shown in Fig. 4a, the density of both the core and interface increase along the folding route ($\dot{\mu}_{\text{int}} > 0$ and $\dot{\mu}_f > 0$). The size of the core increases with Q , but V_f increases more slowly than N_f (see Eq. 7 with $\nu < 1/3$). Similarly, N_{int} and V_{int} both increase with Q throughout much of the growth. At larger Q , V_{int} reaches a maximum and subsequently decreases rapidly as interfacial residues are consumed by the folded core.
- Pattern B (*core consolidation dominated growth*). As shown in Fig. 4b, the growth of the core is similar to Pattern A ($\dot{\mu}_{\text{int}} > 0$), while the density of the interface decreases ($\dot{\mu}_{\text{int}} < 0$). The difference between pattern A and the pattern B growth is that in pattern A the core and interface expand together relatively rapidly, while in pattern B the core grows at the expense of the interface.
- Pattern C (*balanced growth*). As shown in Fig. 4c, the packing fraction of both the interface and core are roughly constant through much of the folding in Pattern C growth ($\dot{\mu}_{\text{int}} \approx 0$ and $\dot{\mu}_f \approx 0$). Here, as the nucleus grows, the interfacial residues incorporated into the folded core are balanced by unfolded residues entering the interfacial region.

The growth mode of the nucleus for the 27 proteins considered in this paper (1pgb16 is too small to have a compact folded core) can be roughly classified as follows: Pattern A (1pgb, 1a0n, 2ptl, 1shg, 1psf, 1pks, 1pin, 1c8c, 1fkb, 1fnf9, 1wit, 1urn); Pattern B (2pdd, 1enh, 1coa, 1vii, 1aps, 1imq, 2abd, 1hdn, 1div); Pattern C (1lmb, 1csp, 1srl, 1ten, 1o6x, 1mef).

Polarized vs diffuse critical nucleus.

A folding mechanism is typically characterized by the structure of the critical nucleus. The spatial structure of the transition state ensemble, inferred from ϕ -value analysis, has often been qualitatively summarized as either diffuse or polarized.²⁸ Intermediate ϕ -values spread across a large portion of the protein sequence indicate a diffuse nucleus. In contrast, polarized transition states are inferred when only one part of structure has relatively high ϕ -values while the rest of the residues have low ϕ -values. In addition to a bimodal distribution of ϕ -values, the ordered residues in a polarized transition state ensemble are located in one region in the native configuration. Polarized and diffuse critical nuclei are sometimes called localized and delocalized transition state ensembles, respectively.²⁹ Of course, the critical nucleus of a given protein is expected to have structural properties somewhere between the two limits of polarized and diffuse. The second row of Fig. 3 gives an example a diffuse critical nucleus (1lmb). For comparison, Fig. 5 shows the corresponding plots for a protein with a polarized critical nucleus (1srl). Comparing with Fig. 3 and Fig. 5, it is clear that the interface of 1lmb is much broader than the interface region of 1srl. Furthermore, the folded core of 1lmb is much more diffuse than the folded core of 1srl.

Characterizing a capillarity-like ordered nucleus as either diffuse or polarized is a statement of the sharpness of interface as well as compactness of the core. For convenience, we monitor both regions by the normalized volume per monomer (inverse packing fraction): $1/\mu_f$ and $1/\mu_{int}$. The results for the two state proteins considered in this work are shown in Fig. 6. Nuclei with small values of $1/\mu_f$ and $1/\mu_{int}$ are more polarized with relatively compact cores and sharp interfaces (similar to those envisioned in the strict capillarity approximation). Diffuse nuclei, on the other hand, have extended regions of partial order which corresponds to large values of $1/\mu_f$ and/or $1/\mu_{int}$. We note that relatively polarized nuclei can have cores that are still loosely packed compared to the native state density (eg., 1pgb). Furthermore, relatively diffuse nuclei can have tightly packed cores but extended interfaces (eg., 2abd, 1imq, 1fkb).

Our predictions for proteins with polarized transition state, such as 1csp⁹, 1srl⁷, 1shg⁶, 1pin⁸ 2ptl³⁰, and 1pgb³¹, are consistent with classification inferred by experimental ϕ -value analysis. Several protein classified as having diffuse nuclei are also predicted by our model, such as 1lmb³², 2abd³³, 1imq³⁴, and 1fkb³⁵. Nevertheless, the predictions are at odds with

experimental measurement for a few proteins. Our model predicts that the folding nucleus of CI2, perhaps the archetype for a diffuse transition state ensemble, is relatively polarized. This is also true for 1aps.³⁶ Another exception is U1A, which has been shown experimentally to have an early, delocalized transition state.³⁷ The calculated folding route from this cooperative model several transition states along the folding path, but the highest barrier corresponds to a late, polarized nucleus. If we look at the earlier transition state ensembles (also shown in Fig. 6) the structure of the nucleus is much more diffuse. The same situation arises in 1pgb,³¹ for which the calculated folding route has two transition states; the early one which is more diffuse has a lower free energy than the late one which is more polarized.

We note that in these exceptional cases, ϕ -value distributions indicate the critical nucleus is rather diffuse while our model predict more polarized nuclei. This tendency can be understood as a consequence of the model being overly cooperative for these proteins, since cooperativity generically tends to sharpen the interface between folded region and unfolded region, and hence is somewhat biased towards polarized transition states.

Conclusion

In this paper, we directly characterize folding in terms of the capillarity-like growth of the folding nucleus. The nature of the partially folded interfacial region between the folded core and unfolded halo is the central focus of characterizing the growth modes of the nucleus. We find that the growth of the nucleus can be classified into three different patterns: (A) the core and interface both condense along the folding route; (B) the core condenses at the expense of the interfacial region; and (C) the growth of the core is balanced by the monomers entering the interfacial region from the unfolded halo. The picture of the core as close packing of rigid monomers appears to be valid on average, though the size of the effective monomers is larger than one would expect for a native-like, compact core. Furthermore, this analysis clarifies that diffuse nuclei inferred by the distribution of intermediate ϕ -values for example can arise from either a diffuse folded core, a broad interfacial regions, or both. The predictions from our calculations can be tested from the analysis of the evolution of ϕ -values as a function of the movement of the transition state ensemble (β^\dagger) pioneered by Oliveberg and co-workers.^{37,38}

The variational model considered here includes a uniform “neutral”, excluded volume

type cooperatively developed to account of general trends in the absolute folding rates of two state proteins.¹³ The exceptional qualitative discrepancies of the the polarized versus diffuse characterization of the critical nucleus (such as CI2, 1aps, 1pgb, and U1A) permit an opportunity to assess the form and strength of the cooperativity of this model. The spatial density of the critical nucleus can be used as an independent criteria to check the value of the cooperativity obtained by simultaneously fitting ϕ -values and barrier height by the parameterization of the cooperativity for each protein. There are some indications that one should consider variations in the strength of the cooperativity for different proteins (though, admittedly this is very closely tied to the specific form of the cooperativity in the model). For example, Ejtehadi and Plotkin recently found that the strength of cooperativity from three-body interactions can be tuned for each protein to bring simulations of ϕ -values into better agreement with experimental measurement.³⁹ Furthermore, detailed analysis from a similar variational model suggests that the cooperativity of the U1A protein is much lower than assumed in this model.³⁸ The generally good qualitative agreement between our calculations and experimental inferences about the spatial extent of folding nuclei suggest that tuning the excluded volume strength for each protein would not greatly improve the results presented here for the majority of the proteins studied.

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Figures

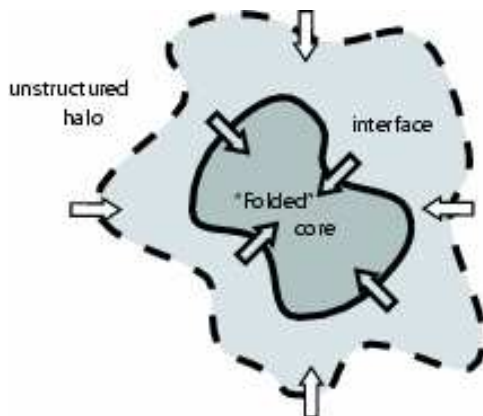


FIG. 1: Illustration of folding nucleus: folded core, interfacial region, and unfolded halo. Growth of the nucleus can be characterized by fluxes entering the folded core and interfacial regions.

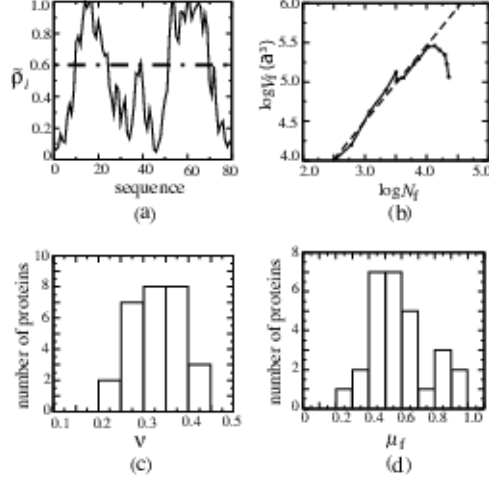


FIG. 2: Scaling of the folded core with number of monomers. (a-b) correspond to λ -repressor (1lmb). (a) Residues with native density $\tilde{\rho}_i > 0.6$ (indicated by the dashed line) define a fiducial set of folded residues. (b) Linear fit of $\log V_f$ vs. $\log N_f$ (dashed line) gives the exponent of $V_f \sim N_f^{3\nu}$. In this example, the fitting equation is $y = 5.6 + 0.97x$, so that $\nu = 0.32$ and $b^3 = 5.6a^3$, $a = 3.8\text{\AA}$ is the average distance between the α carbons. (c) Histogram of scaling exponent ν for 28 proteins; (d) Histogram of the packing fraction of the folded core of the critical nucleus at T_f .

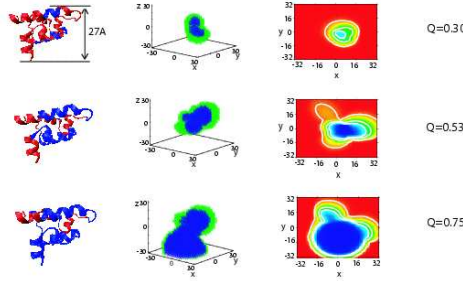


FIG. 3: Illustration of growth of folding nucleus and interface along the folding route (increasing Q) for the λ -repressor protein (1lmb). Column 1 shows the three-dimensional folded structure with the fiducial set of folded residues colored blue and the unfolded residues colored red. In Column 2, the folded core (colored blue) is surrounded by the interfacial region (colored green). Column 3 is a projection of the indicator function $\tilde{n}(\mathbf{r})$ that defines the folded and interfacial regions in space. The values correspond to $\max_z \tilde{n}(x, y, z)$, ranging from 1 (blue) to 0 (red) in steps of 0.01. Contour lines correspond to 0.1, 0.5, 0.7. Column 4 gives the corresponding Q value for each row. The critical nucleus corresponds to $Q = 0.53$. The units for three plots are in Angstroms. This protein is belong to the Pattern C (balanced growth). The three-dimensional structure was produced by VMD.²⁷

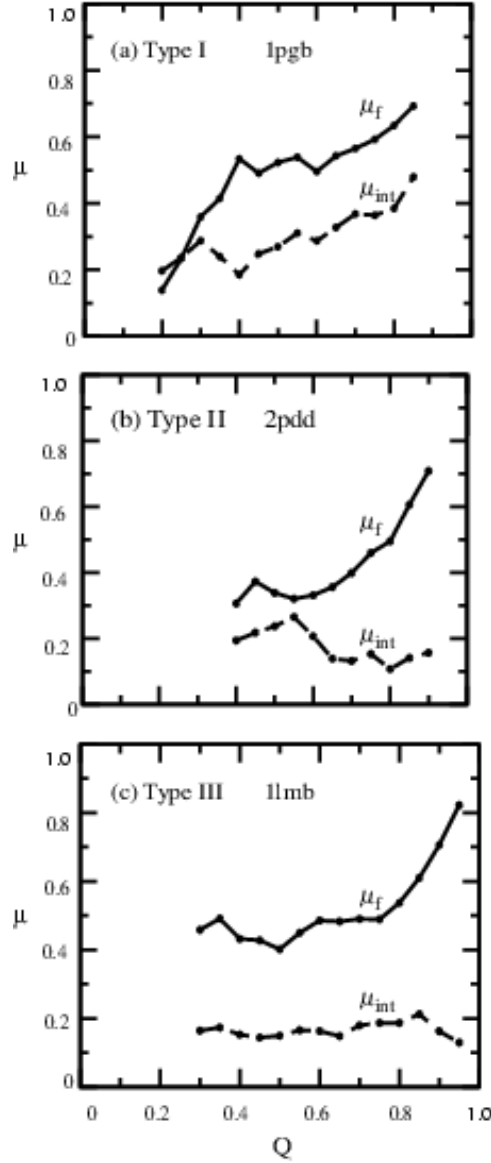


FIG. 4: Examples of three modes of growth of the nucleus. Pattern A–C corresponds to (a–c), respectively. Solid line corresponds to the mean packing fraction of the folded core, μ_f , while the dashed line corresponds to the mean packing fraction of the interface, μ_{int} .

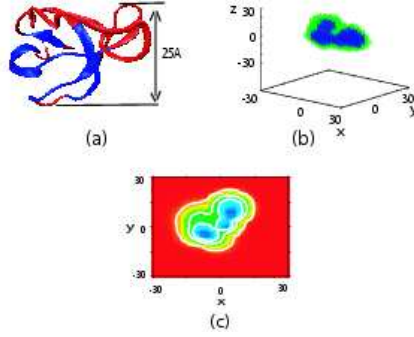


FIG. 5: An example of a polarized critical nucleus ($Q=0.45$) for Src-SH3 (1srl). Plots (a–c) correspond to the middle row of the diffuse nucleus shown in Fig. 3.

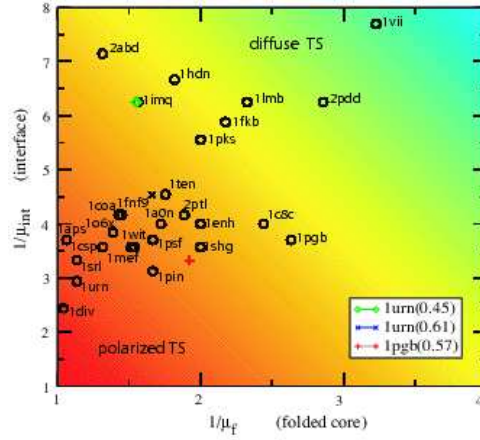


FIG. 6: Inverse packing fraction of the interface and folded core. for 27 two-state proteins. The gradual change color shows the continuous change from polarized nuclei (red) to diffuse nuclei (cyan). Also shown are two early transition states of U1A (1urn) and a late transition of protein G (1pgb).